# Development of the elements of the biological system for protecting crops against the southern green stink bug *Nezara viridula* L. (Hemiptera: Pentatomidae) in Krasnodar Krai

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**Abstract.** The article considers the issues related to the development of the methods of biological crop protection against the adventive species of pentatomides (Pentatomidae, Hemiptera) - the southern green stink bug *Nezara viridula* L. 1758. One of the main factors contributing to the significant spread of this multinucleated pest is the lack of effective control systems of this species, in particular, poor knowledge of local entomophages species. A number of the biological peculiarities of a stinkbug has been studied. For the first time in the Russian Federation, infection of the *N. viridula* eggs with the entomophage *Trissolcus basalis* Woll. 1858 (Hymenoptera: Scelionidae) was determined We found that to control *Nezara* in such crops as soybeans, tomatoes and tobacco, it is possible to use the Fitoverm-M preparation (active ingredient – aversectin-C, 2 g/l).

## 1 Introduction

Currently, agrocenoses are under the constant influence of various environmental factors, both biotic, abiotic, and anthropogenic, associated with the large-scale globalization [1]. This has led to the fact that over the past decade, a number of stinkbugs such as *Nezara viridula* L. 1758, *Halyomorpha halys* Stål, 1855, *Piezodorus guildinii* Westwood, 1837, *Megacopta cribraria* Fabricius 1798, have expanded their habitats and acquired the status of invasive pests in many countries of the world [1, 2]. For example, in a number of regions of Krasnodar Krai, significant damage to various crops such as soybean *Glycine max* (L.) Merr, 1917, solanaceous crops (Solanaceae) (tomatoes (*Solánum lycopérsicum* L. 1753), pepper (*Capsicum annuum* L. 1753), eggplant (*Solanum melongena* L.1753), and tobacco (*Nicotiana tabacum* L. 1753) is caused by the southern green stink bug *N.viridula* [3-5].

Southern green stink bug has been recorded in the Kuban region, according to various sources since 2006 [2, 5]. It should be noted that in South Europe and in America it is spread almost everywhere [6-9]. The serious danger of this pest is that according to available information, it can damage plants of more than 30 families, including cereals

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(Poáceae) (wheat (Triticum durum Desf. 1930), rice (Oryza sativa L.1753), etc. d.), legumes (Fabáceae) (soybeans), technical (cotton (Gossypium herbaceum L.1753)), vegetables (nightshade (Solanaceae), cruciferous (Brassicáceae), pumpkin (Cucurbitaceae), etc., on both closed and open ground, garden crops and shrubs, as well as ornamental plants [8, 10-11]. *N. viridula* propagates itself all year round in the tropics, in the temperate zones, including Krasnodar Krai, this species has a reproductive winter diapause [2].

Nowadays, the chemical method continues to be one of the most effective methods of controlling green vegetable bugs, while using preparations with various active substances, mainly pyrethroids and organophosphorus compounds [11, 12]. Along with the use of chemical pesticides active biological agents that can restrain the abundance of *N. viridula* are being searched for all over the world. Among the biological products against the pest, mainly preparations based on *Beauveria bassiana* (Bals.-Criv.) Vuill. 1912 and *Basillus thuringiensis* Berliner 1915 were tested, their efficacy against larvae and adults up to 90 % [11, 12].

According to the data published by some foreign researchers, hymenopterans (Scelionidae family), egg parasite *Trissolcus basalis* Woll. 1858 in particular, are highly effective against *N. viridula.* There is some evidence of promising studies carried out in Brazil and Spain on the possibility of mass breeding and use of two egg parasites *Telenomus podisi* Ashmead 1893 and *T. basalis*. The efficiency level of entomophages was 80–90 % [13–15].

Thus, the information available in the literature, as well as the data on the constantly growing number of the pest in Krasnodar Krai, indicate the need to develop techniques for an effective system of biological protection against *N. viridula*.

## 2 Materials and methods

The research was carried out on the basis of a ten-field grain-hoed crop rotation of the All-Russian Research Institute of Biological Plant Protection (ARRIBPP) on soybean Arleta and Vilana varieties (0.01 ha plots were selected for testing the biological product) and on Yubileiny Novy 142 tobacco crops and on tomato Gaidas variety in the All-Russian Research Institute of Tobacco , shag and tobacco products (ARRITTP). All the studies were carried out according to the standard methods for the study of pentatomid bugs [16].

Entomophages were detected using the kairomone feeding places (KFP) developed at the ARRIBPP, which are a metal frame with a diameter of 25 cm and a height of 20 cm, covered with mesh material, allowing entomophages to freely penetrate the device, where 20-25 stinkbug imagoes were placed. In order for the bugs not to creep away, KFP was fixed in the upper part with rubber rings. Inside the feeding place, at the bottom, there was one drinking bowl with a moistened cotton swab and feeders with seedlings of mung beans. Using KFP similarly to the pheromone devices, we determined the species composition of Nezara egg parasites [5]. The species affiliation of the entomophages was confirmed by A. Timokhov. (Moscow, M.V. Lomonosov Moscow State University).

Laboratory and field screening of the biological agents was carried out according to the standard methods, biological efficacy was determined by the Abbot formula [5]. For preliminary screening we used the biological agents based on *B. bassiana, B. thuringiensis* and *Metarhizium anisopliae* Sorokin, 1883, as well as Phytoverm-M KE biopreparation, 2 g/l, the active substance of which is a metabolite of soil actinomycetes of the genus *Streptomyces* - aversectin-C. In the field, the effect of the preparations was studied under the conditions of a small-plot experiment on soybean, tobacco and tomato plants against *N viridula* larvae of the $1^{st}$ age and, in part, against the adults of the first summer generation on tobacco plants, of the second summer generation on tomato and soybean plants with a population of 5-10 ind./$m^2$ , plants of the control option were not treated.

For the laboratory studies, we used the following types of entomopathogenic nematodes (EPN) from the families Steinernematidae and Heterorhabditidae - *Steinernema feltiae* Filipiev 1934, *St. kraussei* Steiner 1923, *St. carpocapsae* Weiser 1955 and *Heterorhabditis bacteriophora* Poinar, 1976, discovered by the employees of the institute in various regions of Krasnodar Krai, and received from the collection of the FSBSI VIZR (St. Petersburg) and the Institute of Parasitology and Helminthology RAS. Laboratory testing of suspensions of entomopathogenic nematodes (EPNs) was carried out in Petri dishes, where the *N viridula* imagoes were treated with invasive larvae of EPNs at a dose of 100 ind. on one insect, germinated mash seeds were also placed in the dishes, serving as food for Nezara, and a moist cotton swab, providing favorable conditions for maintaining insects. There were three replications of the experiment (50 bug individuals for each replication). The death of the insects was determined after 1, 3 and 7 days. In the control option, the adult bugs were treated with distilled water.

Statistical data processing was carried out according to generally accepted methods of statistical analysis and using the Statistica v.12.6 program ("StatSoft, Inc.", USA). The tables show the mean (M) and standard error of the mean (± SEM) [17].

## 3 Results and discussion

We began the development of biological protection methods against a green vegetable bug with the study of its landscape-bitopic spreading in the studied agrolandscapes. As shown by the results of route surveys of the ARRIBPP crop rotation, the overwintered generation of *N. viridula* L. began its development on woody plants (for example, mulberry (*Morus alba* L. 1753)), later migrating to such crops as rapeseed, alfalfa (*Medicago sativa* L.1753), then to corn (*Zea mays* L. 1753) and sunflower (*Helianthus annuus* L. 1753), then migrating to soybean. Moreover, we recorded the imago of the bug on winter wheat crops in the heading phase (Z 55-59), however, no visible damage caused by the pest was noted.

On tobacco plants, *N. viridula* imago and larvae of the first summer generation began to damage plants in the field almost immediately after their rooting and all pest stages were observed in tobacco crops until the end of the growing season, damaged plant leaves lost turgor, and they drooped. The next generation migrated to tomatoes, where its number was the largest. Later, after feeding on tomato and soybean crops the pest imagoes of the new, wintering generationt migrated to tree-shrub vegetation, for example, boxwood plants, where they developed till October, and in warm weather (average daily temperature - 15-20 $^0$C) - till November while not mating, and not laying eggs.

An analysis of the references showed that one of the widely used bioagents for controlling the bug is a number of entomophages belonging to different systematic groups, such as hymenoptera (Hymenoptera), hemipterans (Hemiptera), and dipterous (Diptera) [13, 14]. So, we carried out the work to identify the native species of egg parasites of the vegetable bug. As a result, in 2018 with the help of KFP on soybean crops, the colonization of the pest eggs by *T.basalis* species was detected, which infested up to 10 % of the bug eggs, however, the egg parasites flew out of only 3 % of the eggs. In 2019, there was an increase in the number of the infected eggs of *N. viridula* up to 15 % (50 eggs were infected from the total 500 eggs laid in the KFP), however, the percentage of the released entomophages did not exceed 5 %, which is not enough to control the number of the pest.

Since local entomophages did not inhibit the growth of Nezara, we previously conducted laboratory screening of biological products available in Russia based on entomopathogenic microorganisms and their metabolites [5]. As the results of our studies showed, the preliminary biological efficacy of Phytoverm-M, at a concentration of 0.3 %, was 97.1 % and it was almost similar to the chemical standard - the BI -58 New CE preparation (dimethoate), 400 g/l (BASF- CE) — 98.6 %.

Under the laboratory conditions, we also tested suspensions of entomopathogenic nematodes, since this group of pathogens is one of the most effectively used to regulate the number of pests, and specifically pentatomide [18]. We found that 3 days after infection with nematodes, up to (75.2 + 3.4 %) - (84.3 + 2.7) % of the *N viridula* imago died (Table 1).

**Table 1.** The entomopathogenic nematodes efficacy against the green vegetable bug *N.viridula* under the laboratory conditions (ARRIBPP, 2018-2019)

| Used nematodes | The death of the larvae 3 days after the treatment, % M + SEM, | The number of invasive larvae of entomopathogenic nematodes released from one insect, M + SEM, x $10^4$ pcs., |
|---|---|---|
| *St.carpocapsae* | 84,2±3,1 | 2,7± 0,9 |
| *St.feltiae* | 84,3±2,7 | 2,7±0,8 |
| *St. krausei* | 75,3±2,2 | 0 |
| *H.bacteriophora* | 75,2±3,4 | 0 |

It should be noted that we recorded the isolation of nematodes only in the case of using two types of *St. carpocapsae* and *St. feltiae,* the number of released invasive larvae of EPN was (2.7+ 0.9) × 104 ind. per insect (table1). The obtained data allow us to carry out further research.

For field trials, we chose the bio-insecticide Phytoverm-M, as it showed the best results under the laboratory conditions. The results in table 2 show, that the use of the Phytoverm-M insecticide at a consumption rate of 0.5 kg/ha led to a decrease in the number of bug larvae by (87.5 + 2.6) %, which did not significantly differ from the efficacy of the option treated with Bi-58 New - (90.0 + 3.0) %, the preparation efficacy against the imago was (76.8 + 3.3) % compared with (80.0 + 2.8) % in the standard.

**Table 2.** Biological efficacy of the Phytoverm-M, CE (0.2 g / l) insecticide against the bug *N. viridula* L. on tobacco, tomatoes and soybean (ARRIBPP, ARRITTP, 2019)

| Preparation | Death of larvae, %, M+SEM, in | | Biological efficacy, % M+SEM, | Death of imago, %, M+SEM, inз | | Biological efficacy, % M+SEM, |
|---|---|---|---|---|---|---|
| | 7 days | 14 days | | 7 days | 14 days | |
| Phytoverm-M, 0,5 kg/ha | 91,3±1,5 | 1,5±0,3 | 87,5±2,6 | 79,4±2,6 | 4,1±0,8 | 76,8±3,3 |
| Bi-58 New, 1,2 l/ha (standard) | 96,3±2,2 | 1,2±0,1 | 90,0±3,0 | 84,3±1,8 | 4,0±1,1 | 80,0±2,8 |
| Control (without treatment) | 1,0±0,2 | 5,1±1,4 | - | 4,2±1,5 | 2,8±0,6 | - |

Moreover, we noted that the use of Phytoerm-M resulted in the fact that the surviving females of *N. viridula* did not have oviposition. In general, the use of biological products reduced the damage to tobacco, tomato and soy plants.

Thus, our studies have shown the possibility and prospects of using biological agents to protect crops against the adventitious species of the *N. viridula* bug, the most effective of which was the preparation based on actinomycete metabolites - Phytoverm-M. The fact of the first detection in the Russian Federation of such an entomophage egg-parasite as *T. basalis* is also significant, however, the study of the peculiarities of its development under natural and laboratory conditions must be continued in the future.

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